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Editor

FREDRICK J. STARE, PH.D., M.D., Associate Professor of Nutrition, Schools of Medicine and Public Health, Harvard University; Associate in Medicine, Peter Bent Brigham Hospital, Boston. Editorial Office, 25 Shattuck St., Boston, Mass.

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NUTRITION REVIEWS

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TRENDS IN NUTRITION RESEARCH AND APPLICATION

Until the twentieth century, research consisted largely of individual, independent investigation within restricted channels. Each scientist pursued, in his chosen field of endeavor, the approach which he deemed most productive. There were clearly defined demarcations between chemistry and physics, physiology and anatomy, economics and sociology. Any field of endeavor offered unlimited opportunity for research, without conflict with other workers. Progress was slow, but gradually the fundamental truths came to light. As our knowledge multiplied it became possible to "specialize" in a particular phase of one of the fundamental or applied sciences; research became an even more isolated pursuit. However, the knowledge produced by specialization was not sufficient, in itself, and new sciences arose to bridge the gaps between two or more of the parent sciences. And as the horizons of research lifted, the necessity was recognized of integrating the objective results of the laboratory and social sciences, not only with each other, but also with subjective results obtained by the mental sciences.

Today the science of nutrition utilizes the combined knowledge of fundamental and applied sciences. Nutrition research is conducted in the laboratories of hospitals, schools, agricultural experiment stations, and food manufacturers—with human subjects, animals, plants, and microorganisms—by chemists, physicists, biologists, physiologists, bacteriologists, biometricians, physicians, home economists, dietitians, sociologists, economists, and agriculturists. Scientists working in different specialties have pooled their efforts to obtain more complete knowledge of the foods we eat and of the physiologic processes that utilize them. Indeed, in its development, application, and progress, the science of nutrition embraces most, if not all, of the sciences.

At the turn of the century, students of nutrition believed the digestible parts of natural foods consisted only of protein, carbohydrates, fats, and minerals. These constituents could be determined in the chemical laboratory and the results of such analyses indicated that nearly all of the food material used in the body was accounted for as protein, fat, carbohydrate, and ash. During the next two decades "vitaminology" was born and flourished. Because of their practical significance, vitamin discoveries stimulated the most intensive period of biologic research of all time, heralding "the science of man" which is being so intensively and successfully pursued today. Interest in the amino acids in the past few years has made these protein constituents rivals of the vitamins in nutrition research. Also during the past twenty years the term "minerals" has been recognized as including some eighteen to twenty materials essential for the development and maintenance of healthy body structure. Essential fatty acids, hormones, and other constituents contribute to physiologic activity and intermediary metabolism.

It no longer suffices to know that healthy human bodies of a certain age can assimilate a maximum or minimum amount of calcium, will not grow without a definite amount of phosphorus, or will show symptoms of deficiency without adequate vitamin intake. As such factors have become clearer, the information has opened new vistas. Today we know that an adequate intake for one child may be inadequate for another, that at different ages and in different climates re-

quirements may differ, and that physical status, mental health, and past experience may influence body needs. As yet we do not have all the criteria by which to classify these individual variations—nor the methods with which to derive them, but they will be necessary for the science of nutrition to keep pace with the development of our civilization. Likewise, it is becoming increasingly urgent that we know more fully the variations in nutritional requirements coincident with and consequent to all forms of morbidity. Evidence in the past few years has shown that nutrition research offers many opportunities in the direction of collateral therapy, as well as on behalf of preventive medicine. It is probable that in the course of studying the requirements for good nutrition—good health—research will uncover the causes of conditions which now receive only symptomatic treatment.

Today, nutrition research on man faces the task of determining nutritional requirements on the basis of total intake of all essential materials. The requirements for "materials" must be translated into terms of food, food handling, and healthful living, include provision of "safety factors," yet avoid waste. They must be established for infants, children, adults, and the aged; for the sick, the well, and the handicapped; and for different races and climates. For any group the needs must be met by the amounts and types of food which can be made available.

Brozek and Keys (*Science* **100,** *507 (1944)*) emphasized that investigation of the intact human organism is dependent upon an "interdisciplinary" approach, which in turn is dependent upon the cooperative efforts of investigators with intensive training in a specialized field and broad training in science in general. These authors demonstrated the revisions which must be made in the training programs of our universities to secure greater numbers of scientists who will be capable and enthusiastic workers in the larger problems which can be solved only by interdisciplinary research. As knowledge in the many individual sciences is amassed, the need for integrated, comprehensive investigations of problems relating to the health of our population is greatly increased and yearly becomes more urgent.

Beyond recognition of the value of broad, integrated approaches to problems of nutrition and the special training of personnel for interdisciplinary research, there are practical considerations involved in extensive investigations. It is not surprising that wide application of these methods has been achieved first by industrial and commercial laboratories, wherein financing and administration are usually directed toward eventual profits. In the so-called "pure research," which has only limited possibilities of direct application but may have greater intrinsic value, programs are frequently inhibited in concept or limited in execution by lack of assurance of adequate financing, both initially and throughout the months or years which may be required to complete the project. Fortunately, in the past decade government, education, business and private interests have become increasingly aware of this problem and in many instances have made funds available for research of this type.

We are approaching several objectives: universal recognition of the advantages of group research; training programs to develop the personnel most suitable for cooperative endeavors; financial support to assure their execution and the compilation, interpretation, and presentation of the results; and administration capable of planning productive investigations and conducting them efficiently. As these objectives are gained, research in nutrition will be accelerated and the lag between the production and application of experimental results will be shortened.

Icie G. Macy,
Director, Research Laboratory,
Children's Fund of Michigan,
Detroit, Michigan.

FACTORS IN THE CONTROL OF NUTRITIONAL EXPERIMENTS ON HUMAN SUBJECTS

The National Research Council's Committee on Diagnosis and Pathology of Nutritional Deficiencies has published a somewhat lengthy report entitled "Principles Underlying Studies of Nutrition Pertaining to the Influence of Supplements on Growth, Physical Fitness and Health," (*Arch. Int. Med.* **74,** *258 (1944)*) which is timely in drawing attention to some rather obvious, but important, principles. The basic point made by the authors is that there are ten conditions governing such studies on human subjects which must be controlled and separately evaluated before the studies can themselves be evaluated or compared with one another.

The first condition is the index or criterion for appraising the nutritional status of the subjects. This must be appropriate and adequate. Growth, in its usual meaning of increase in body weight, is rightly criticized as such an index; but on the other hand the authors apparently subscribe to the view that any increase in growth rate indicates an improvement in nutritional status, a view which is also open to criticism.

The second condition is the nutritional status of the subjects at the beginning of the experiment. It would of course be desirable to know this as accurately as possible but the authors omit mention of the difficulty that no general agreement can yet be obtained on how to measure the nutritional status of the subjects. This is by no means a bar to experimentation however, since the observer is justified in describing the condition of the subjects to the best of his ability, and noting all changes in their condition during the course of his experiment. It is only from the records of much more work of this type that we may hope to learn how to assess nutritional status.

The third condition is the diet of the subjects both before and during the study. The fourth condition is the operation of conditions which produce malnutrition "by influencing the relation between supply and bodily requirements" of nutrients. We are told that "such operation, amounting to uncontrolled change in the conditions of a study, affects its results. Therefore these conditions have an influence either favorable or unfavorable not only on nutrition but on the course and outcome of a study. But the direction of their effect is not necessarily the same on one as on the other. They may operate adversely on the outcome of the study by producing either a beneficial or a detrimental effect or by preventing a beneficial effect on nutrition. Likewise, they may operate favorably on the outcome by either an advantageous or a deleterious effect on nutrition."

The fifth condition is set by the indices of growth, physical performance, reaction to infection, or whatever else is measured by the observer. The sixth condition is the status of the subjects at the outset with regard to growth, physical performance, and immunity and resistance to disease. The seventh is the method of selection of the subjects for study, and their relation to the population at large. It is pointed out that one cannot generalize from a sample of the population to the population at large unless the sample is drawn without bias.

The eighth condition is the number of subjects studied. The ninth is the nature and potency of the dietary supplements given to the subjects, and the tenth is the term of the experiment.

It will be seen that these conditions do indeed effect the outcome of an experiment the purpose of which is to determine the effect of a dietary supplement, and that only rarely can they all be controlled. One may however question the necessity of a long discussion of the thesis that "the nature and potency of the supplements bear on the results. The expectation of the authors that recognition and evaluation of these

conditions in studies from which at the present time conflicting conclusions are drawn will make "conflict and confusion give way to conformity and clarity" must be regarded as altogether too optimistic. There are other conditions just as important as these ten, and instead of enumerating them all, a strong plea for the use of common sense and critical judgment in the setting up of nutrition experiments on human subjects might be more effective. No doubt the Committee has attempted to outline the basis for critical judgment in such studies.

The report contains a comprehensive bibliography on supplementary feeding which should prove to be of distinct value.

It is particularly important to see that suitable experimental controls are used, as was stressed again by Muench (*Proc. Res. Conference on Relation of Nutrition to Public Health, p. 45, The Nutrition Foundation, New York (1943)*). In view of the psychologic factor, it is essential that any group of subjects given a food supplement shall be matched by another group given a placebo which in appearance and taste is not distinguishable from the supplement whose effect is to be tested. Further, it is equally essential that neither the subjects nor the observers recording the results shall know which subjects receive the test supplement and which the placebo. One individual alone should have this information, and he should be the one who is responsible for distribution of the supplements to the subjects, and for recording their consumption. Only in this way can subjective influences on the findings be reduced to a minimum.

Then again there must be a rational attitude toward the indices used in assessing the condition of the subjects before and after the supplementary feeding. If the index is a new one, not yet widely tested, that fact should be plainly stated and the conclusions drawn from its use should be regarded as tentative. A new index or method is not better than an old one until it is proved so. When there is evidence which strongly suggests that an index is unsuitable or unreliable then it should be discarded. Such an index for instance is the condition of the gums, often taken as a criterion (sometimes as the sole criterion) of nutritional status with respect to ascorbic acid. A pertinent review (*Nutrition Reviews* **2,** *44 (1944)*) cited clinicians of the past and experimenters of the present time who established the view that while diseased gums may be due to ascorbic acid deficiency, more often they are not.

Here then are two more conditions of human nutrition experiments which must be watched and controlled, and subjected to rational criticism, if the results of the experiments are to be sound and capable of being embodied in a mass of sound scientific doctrine in harmony with the results of other similar experiments. For, just as the condition of liberty is eternal vigilance, so also the condition of soundness in scientific doctrine is eternal vigilance, in the form of rational criticism of experiment and inference alike.

THE AMINO ACID REQUIREMENTS OF THE CHICK

Early attempts to determine the amino acid requirements of the chick were made by feeding proteins known to be deficient in specific amino acids, or by feeding protein hydrolysates from which one or more of the original amino acids present had been removed or destroyed. With these methods it has been shown that the chick must be supplied with dietary sources of arginine, glycine, histidine, tryptophane, lysine, methionine (Almquist, *Federation Proceedings* **1,** *269 (1942);* Almquist and Grau, *J. Nutrition* **26,** *631 (1943)*), and isoleucine (*Poultry Sci.* **23,** *488 (1944)*). The usefulness of such

comparatively crude diets, however, was limited by the incompleteness of the deficiencies that could be produced by them, and by the lack of sufficiently sensitive tests to indicate the composition of the amino acid mixture actually supplied to the bird.

The classic experiments of Rose (*Physiol. Rev.* **18,** *109 (1938)*) indicated that the rat will grow on diets containing amino acid mixtures as the sole source of nitrogen, and that ten amino acids are required for the growth of this species. Rose defines an indispensable amino acid as "one that cannot be synthesized by the animal organism out of materials ordinarily available at a speed commensurate with the demands for normal growth." Similar experiments on chicks, however, have been delayed, because of the complex nutritive requirements of this species. Results of experiments with crude rations, for instance, had indicated that the chick needs much more glycine and arginine than the rat, and when attempts were made to feed amino acid mixtures in purified diets, natural feedstuffs had to be added in order to produce an acceptable growth response.

More recently, Almquist and Grau (*J. Nutrition* **28,** *263, 325 (1944)*) have succeeded in preparing a basal ration which will support growth in the chick and which contains only 0.004 per cent of uncharacterized nitrogen. Crystalline amino acids supplied virtually all of the dietary nitrogen. This ration consisted of cellulose 5, calcium gluconate 8, mineral mixture 3.24, crude soybean oil 5, amino acid mixture 30, adequate levels of the fat soluble vitamins furnished as sardine oil, mixed tocopherols, and 2-methyl-1,4-naphthohydroquinone diacetate, and the crystalline water soluble vitamins with glucose to make 100 g. The uncharacterized members of the vitamin B-complex were provided by a concentrate prepared from solubilized liver.

All of the amino acids in this ration were commercial products. When a naturally occurring l-isomer could not be obtained in pure form, the corresponding dl-mixture was fed at such levels that the l-isomer was present in amounts equal to or greater than the currently accepted requirements. Sodium bicarbonate was added to neutralize the hydrochlorides of the basic amino acids. The amino acid mixture consisted of alanine, arginine, aspartic acid, cystine, glutamic acid, glycine, histidine, hydroxyproline, isoleucine, leucine, lysine, methionine, norleucine, phenylalanine, proline, serine, threonine, tryptophane, tyrosine, and valine. When incomplete mixtures were fed, the amino acid omitted was replaced by an equal weight of glucose.

Newly hatched chicks were maintained on a commercial type chick mash for ten to fourteen days. They were then segregated into groups of 2 to 5 chicks and housed in electrically heated wire floored battery brooders. Rations were fed ad libitum for periods of four to twenty-seven days, and the rate of gain expressed as the percentage of the average body weight gained daily. Another method of expressing results was the so-called efficiency of gain (grams gain/grams feed consumed) adopted because some chicks did not eat the ration readily for the first day or two. Over a ten day period the group receiving the complete basal ration gained at a rate of 4 per cent daily of its average body weight.

The removal of alanine, aspartic acid, norleucine, or serine individually from the ration had no effect on growth, nor did the removal of proline and hydroxyproline produce a lasting effect. It was concluded that these amino acids are dispensable for the chick.

Results with 4 chicks fed a glycine deficient amino acid mixture for eighteen days showed poor growth and poor gain per gram of food eaten. The omission or inclusion of serine had no effect on the glycine deficiency. These results confirm the previous conclusion that the chick can synthesize glycine but not at a rate high enough to allow best growth.

When glutamic acid was removed from

the amino acid mixture, 3 chicks showed an original loss in weight and a slow gain thereafter, and the addition of aspartic acid did not alter this result. Accordingly it was suggested that glutamic acid is probably not required for growth to take place, but that it may function in promoting most efficient growth.

Four chicks which received a histidine deficient ration lost weight for the first four days of the experiment and remained at a low weight level for the remainder of the ten day period. Histidine was therefore considered as indispensable. Further results indicated that the requirement for growth is met when the percentage of l(+)-histidine in the diet is 0.15 per cent or more.

Weight loss and death resulted when 3 chicks were fed a ration deficient in isoleucine. When 3 chicks were maintained for sixteen days on a leucine free ration, there was a rapid weight loss followed by a more gradual loss. The requirement of l(−)-leucine appears to lie between 1.0 and 2.0 per cent of the diet.

Results indicate that tyrosine is required to promote optimum growth if phenylalanine is present at only a moderate level (1 per cent) in the ration, but that tyrosine is dispensable when higher levels (3 per cent) of phenylalanine are fed. Thus the relationship between phenylalanine and tyrosine is much the same in the chick as in the rat. When 1.0 per cent of dl-phenylalanine was present, normal growth resulted. The complete omission of dl-phenylalanine from an amino acid mixture containing 2.0 per cent of tyrosine resulted in a rapid loss of weight.

A level of 2.0 per cent of dl-threonine was necessary for adequate growth and therefore it is considered to be an essential amino acid. The removal of valine from the ration resulted in a rapid continuing decline in weight. Three of 4 chicks on one experiment died on the twelfth day and all were extremely weak before this time. The additions of 2.0 per cent of norvaline and 2.0 per cent of isovaline did not diminish the severity of the deficiency. When valine was added after six days all the chicks gained weight within twenty-four hours and continued to do so.

Tryptophane has long been known to be an essential amino acid for the chick, and Almquist and Grau have shown that the dl-isomer is only one-half as efficient in this species as the l-isomer. This deficiency was not only apparent on diets containing mixtures of amino acids, but also on diets containing suitably supplemented zein and gelatin. The maximum efficiency of gain was reached in both types of diets when 0.2 to 0.3 per cent of l(−)-tryptophane was present. Apparently the higher requirement (0.5 per cent) indicated in earlier experiments was for tryptophane which had been racemized. These results indicate a further difference in the amino acid requirements of the chick and the rat, for the latter apparently utilizes both isomers of tryptophane for growth. In the mouse, however, less rapid growth is obtained with the unnatural form than with the natural form.

The conclusions of Almquist and Grau can be summarized in the statements that alanine, aspartic acid, hydroxyproline, proline, norleucine, and serine are dispensable for the growth of the chick; that leucine, phenylalanine, threonine, valine, histidine, isoleucine, and tryptophane are necessary dietary components to prevent weight loss; that glycine and glutamic acid are essential in promoting best growth; and that tyrosine is essential under certain conditions.

Hegsted (*J. Biol. Chem.* **156,** *247 (1944)*) has reported experiments which are in general agreement with those of Grau and Almquist, but on diets containing only the amino acids required by the rat plus glycine and cystine. However, he points out that normal growth has probably never been obtained on diets in which the sole source of nitrogen was supplied by amino acids. He believes that failure to obtain better growth can scarcely be attributed to lack of nitrogen for the synthesis of nonessential

amino acids nor to the fact that the amounts of amino acids fed were suboptimal. A toxicity of the amino acids at this level must be considered but appears less likely than the possibility that the chick has limited synthetic powers for one or more of the amino acids not supplied. The studies of Almquist and Grau show that this is true with regard to glutamic acid. One limiting factor in the experiments of these workers may have been the amount of phosphorus fed, 0.49 per cent of the ration. The level of phosphorus needed by the chick is usually considered to lie between 0.5 and 0.8 per cent of the ration.

Hegsted suggests that the present classification of essential amino acids by Rose throws undue emphasis upon those acids known to be indispensable. The rat can grow in the absence of cystine and to this extent cystine is nonessential but in the absence of sufficient methionine, cystine is fully as important as the so-called essential amino acids. Thus cystine may be an important amino acid in practical nutrition, and a similar situation with regard to certain other so-called nonessential amino acids is possible. Finally, Hegsted points out the possibility that some of the now accepted essential amino acids may be replaceable or interchangeable with other amino acids. He suggests that the essential amino acids be defined as those essential for life.

Growth and maintenance of positive nitrogen balance have been most frequently used as criteria in deciding whether or not an amino acid is essential. Rose and Rice (*Science* **90,** *186* (*1939*)) have stated that glycine and arginine are not required for normal growth by the dog, yet it has been reported by Madden and co-workers (*J. Exp. Med.* **77,** *277* (*1943*)) that arginine is essential for blood regeneration, and that glycine is necessary for plasma protein regeneration in that species (Madden, Woods, Shull, and Whipple, *Ibid.* **79,** *607* (*1944*)). Arginine and histidine are not needed for the maintenance of a positive nitrogen balance in man; however, Holt and co-workers (*Federation Proceedings* **1,** *116* (*1942*)) have reported that in the absence of arginine, the sperm count is decreased and motility greatly reduced. Albanese, Holt, Frankston, and Irby (*Bull. Johns Hopkins Hosp.* **74,** *251* (*1944*)) report that in the absence of histidine an abnormal metabolite, a chromogen, appears in the urine. Certainly the physiologic significance of the individual amino acids is worthy of further consideration.

STUDIES ON THE PRODUCTS OF HUMAN PLASMA FRACTIONATION

The concerted efforts of many independent but integrated groups of workers in the field of plasma and blood transfusion have resulted in the accumulation of much information which has now been published. Collaboration characterizes much contemporary research owing to the complexity of the many special technics required to elucidate the difficult problems upon which attention is focused. The fruits of such cooperative ventures are apparent to everyone who is familiar with the solution of certain problems whose urgency was suddenly manifest when the nation found itself at war. The group of papers discussed in this review is an excellent example of the striking advance which results from a well organized attack from many vantage points upon a specific problem.

Cohn and co-workers (*J. Clin. Invest.* **23,** *417* (*1944*)) begin the discussion with a review of the basic work on characterization of the protein fractions of human plasma and an outline of the theme of the symposium: "How blood plasma, fractionated into its component parts, may be used with the maximum effect in the treatment of clinical conditions." Plasma has

been used with varying degrees of success in the treatment of shock, burns, diseases where a depleted fraction is restored, and as a prophylactic or therapeutic agent for certain infections. Since different constituents serve different functions, it is important for reasons of therapeutic specificity and of economy to find out what parts are best fitted for each special condition.

Homogeneity and preservation of the more labile components were the criteria of successful fractionating and the aim was to concentrate large therapeutic activity into very small fractions. The division of plasma into various fractions has been extended by subdivision of each. Fraction I contains most of the plasma fibrinogen, Fractions II and III contain most of the gamma globulins (immune bodies, isoagglutinins, and prothrombin), Fraction IV contains most of the alpha globulins, and Fraction V most of the albumin.

Williams and co-workers (*J. Clin. Invest.* **23,** *433* (*1944*)) have undertaken electrophoretic and ultracentrifugal studies of solutions of human serum albumin and immune serum globulins obtained from many sources. Albumin has been concentrated to a value of 98.5 per cent from an initial value of 55 to 60 per cent in plasma. Immune globulins have had their gamma globulins increased to 87 to 95 per cent, compared with an initial value around 11 per cent.

Brand, Kassell, and Saidel (*J. Clin. Invest.* **23,** *437* (*1944*)) discussed the amino acid composition of normal human serum albumin, the human gamma globulins of importance in immunity, a fraction rich in beta globulin and human fibrinogen, and fibrin. Similar studies were made on the serum albumin of cattle and the horse for comparison. The most striking differences in distribution were the high percentage of hydroxy amino acids in the gamma globulins, low tryptophane in albumins, and the absence of methionine in horse serum albumin. These studies indicate that the further characterization of the amino acid distribution of plasma proteins will be of vital importance in the field of nutrition.

Scatchard and co-workers (*J. Clin. Invest.* **23,** *445* (*1944*)) concluded that the thermal stability of human serum albumin is such that solutions of pH 6.8 ± 0.2 in 0.3 M sodium chloride do not need to be kept cold unless they are to be stored for a long time or are to be subjected at a later time to temperatures well above 100°F. (38°C.).

Ballou, Boyer, Luck, and Lum (*J. Clin. Invest.* **23,** *454* (*1944*)) reported that various nonpolar unions enhance the thermal stability of human serum albumin, an effect which increases with lengthening of the fatty acid anion.

Scatchard, Batchelder, and Brown (*J. Clin. Invest.* **23,** *458* (*1944*)) investigated the osmotic pressure of plasma and serum albumin at 25° C. over ranges of concentration and pH much wider than those encountered physiologically. By extrapolation they found the molecular weight of albumin to be 69,000, that of plasma to average about 90,000, corresponding to an average weight of about 170,000 for the 40 per cent of the protein which is not albumin. The osmotic pressure-concentration ratios increase rapidly with increasing concentration. They concluded that about 18 ml. of fluid is held in the blood stream by 1 g. of albumin, by 1.2 g. of plasma protein, or 20 ml. of the current Red Cross citrated, pooled plasma.

Janeway and co-workers (*J. Clin. Invest.* **23,** *465* (*1944*)) contributed a report on clinical use of concentrated human serum albumin, including reports of many carefully studied cases. The standard Army and Navy package, 25 g. of albumin in 100 ml. of diluent, is equivalent in osmotic effect to 500 ml. of citrated plasma. Properly processed albumin is innocuous though pyrogenic reactions occurred in some lots which were reprocessed. Albumin was assimilated by hypoproteinemic patients and was not excreted in the urine unless pro-

teinuria had existed previously. Very large doses were required in chronic cases. The effect was transient in hepatic cirrhosis, and nephrotic patients appeared to excrete most of the injected albumin.

Cournand and co-workers (*J. Clin. Invest.* **23,** *491* (*1944*)) compared concentrated human serum albumin, whole blood, and rapidly infused saline in the treatment of 12 persons with shock. Injected albumin was retained for at least six hours except in the presence of hemorrhage or when plasma was escaping into peritoneum or burned tissues. It increased right auricular pressure, arterial pressure, and cardiac output, facilitating recovery from shock. Albumin effected relatively larger cardiac output than did whole blood during recovery from shock. When hemodilution occurs in shock from skeletal trauma or hemorrhage, increased cardiac output is compensatory. Anemia persisting after albumin therapy indicates the need for subsequent transfusion of whole blood. Substantially the same effects were reported on 6 patients treated by Warren, Stead, Merrill, and Brannon (*Ibid.* **23,** *506* (*1944*)).

The concentrations of certain antibodies in globulin fractions derived from human blood plasma were detailed by Enders (*J. Clin. Invest.* **23,** *510* (*1944*)). Plasma Fractions II and III (alpha, beta, and gamma globulins) contain a large proportion of the antibodies for pathogenic bacteria and viruses, and the isoantigens of the human blood groups concentrated four to ten times. The gamma globulins in Fraction II contain antibodies concentrated fifteen to thirty times and often comparable in strength to convalescent sera, reacting with diphtheria toxin, influenza A virus, mumps virus, and the H antigen of E. typhosa. The antibody for 0 typhoid antigen was recovered in large amounts from Fraction II-1. Sixty-two preparations of Fraction II from plasma collected in various parts of the United States showed generally uniform potency except that influenza A antibodies increased following an epidemic.

Stokes, Maris, and Gellis used gamma globulin (normal human immune serum globulin) in the treatment and prophylaxis of measles (*J. Clin. Invest.* **23,** *531* (*1944*)). Fractions II and III were used in only 8 cases but Fraction II in 53. Doses ranged from 5 to 35 ml. and ages from 10 months to 34 years. The disease appeared to be modified in 13 of 31 patients injected after onset of rash. In 24 of 31 patients injected after Koplik's spots appeared but before the appearance of rash, the disease seemed to be modified. In 2 cases no rash developed. Ordman, Jennings, and Janeway (*Ibid.* **23,** *541* (*1944*)) found that normal human gamma globulin afforded protection in 71 per cent and modification in 27 per cent, with 2 per cent failure, among a controlled group of 62 cases with exposure within the family. No significant reactions occurred. Only 7 per cent of 46 uninoculated controls failed to contract measles.

Pillemer and co-workers (*J. Clin. Invest.* **23,** *550* (*1944*)) separated and concentrated isohemagglutinins from group specific human plasma obtaining a specific and stable material with activity sixteen times that of plasma. DeGowin (*Ibid.* **23,** *554* (*1944*)) reported tests of such material by a panel of 11 experts.

Edsall, Ferry, and Armstrong (*J. Clin. Invest.* **23,** *557* (*1944*)) took up the problem of the proteins concerned in the blood coagulation mechanism. The most important were (1) fibrinogen from Fraction II, which can be further purified and (2) thrombin, prepared from prothrombin in Fraction II-2 by conversion with human placental thromboplastin. Fraction I, purified fibrinogen, and thrombin were prepared in dry, stable, and sterile form. Some of the uses of such substances were detailed by Ferry and Morrison (*Ibid.* **23,** *566* (*1944*)). Fibrin films and fibrinogen plastics were prepared in a wide variety of shapes and sizes. Their mechanical characteristics, depending

largely upon the proportion of protein in the solid mass, could be altered to simulate natural anatomical structures, and their susceptibility to proteolytic enzymes could be modified. This was also true for their rate of absorption. Morrison and Singer (*Ibid.* **23,** *573* (*1944*)) were able to regulate the persistence time and thus direct a rational use of these substances for various specific purposes. One of the applications of this new human plastic was reported by Dees (*Ibid.* **23,** *576* (*1944*)). By simultaneous injection of solutions of fibrinogen and thrombin into the renal pelvis at operation, small and elusive stones became enmeshed in the coagulum which was withdrawn through the usual pyelotomy incision. Hawn, Bering, Bailey, and Armstrong (*Ibid.* **23,** *580* (*1944*)) observed uses of fibrinogen and thrombin in the surface treatment of burns which indicate the absence of harmful effects in man. Preformed fibrin films were used in a small series of second and third degree burns. Use of fibrinogen-thrombin roll bandages as an expedient and practical dressing for burns in the field was suggested.

Bering (*J. Clin. Invest.* **23,** *586* (*1944*)) discussed fibrin foam as a hemostatic agent. The potentialities of an absorbable tampon with thrombin activity for hemostasis where conventional methods are unsatisfactory is of manifest interest. In the field of neurosurgery, where bleeding has always presented problems, fibrin foam has already been found to shorten operations and make some successful which would have been impossible without it, according to Bailey and Ingraham (*Ibid.* **23,** *591*, *597* (*1944*)). Its use in the repair of defects in the dura, and in prevention of adhesions between damaged nervous tissue and adjacent structures was related.

Cannon, Humphreys, Wissler, and Frazier (*J. Clin. Invest.* **23,** *601* (*1944*)) studied the effect of feeding possible blood substitutes on serum protein regeneration and weight recovery in the hypoproteinemic adult rat. The efficiency of protein was evaluated by its capacity to increase the concentration of serum proteins and to produce a gain in weight within a seven day period. The rat was used because of its relatively small requirements and because enough could be used to compensate for individual variations. Bovine serum gamma globulin compared favorably with dehydrated beef and with protein from corn germ as an efficient protein builder. Crystallized bovine serum albumin was less effective. The incomplete proteins gelatin and isinglass were much inferior.

When large quantities of human plasma are no longer readily available it is possible that the knowledge developed from this type of investigation will enable us to find substitutes, or may even have gone so far in characterizing the properties of the useful fractions of plasma that bovine plasma fractions may be used or synthesis of particular components will be within reach.

NUTRITION SURVEY OF A RURAL COUNTY IN NORTH CAROLINA

The nutrition survey has come into increasing popularity in recent years in attempts to determine the incidence, type, and degree of various forms of malnutrition. Technics have varied so that the completeness and accuracy of data are not often comparable. Generally there is a wide discrepancy between the somewhat low incidence of clinically recognized deficiency disease, on the one hand, and the widespread use of diets far below the standards set by the Food and Nutrition Board and the prevalence of biochemical evidence suggesting inadequate vitamin intake, on the other hand.

Milam and Anderson (*South. Med. J.* **37,**

597 (1944)) have reported on a nutrition survey in Wayne County, a prosperous sandy loam farming region in the mid-eastern part of the coastal plain of North Carolina. It was selected because it was representative of the better farming areas in which a program of nutrition improvement might be financed by local means if its need was demonstrated by the survey. The surveyed population included almost 4 per cent of the rural elements of the population, to which the survey was confined. Two hundred families (40 per cent Negro in a county with about 43 per cent of the population Negro) were selected in order to get a representative sample of the population comprised of owners, tenants, and sharecroppers. In addition to the methods these authors had used previously (*Am. J. Pub. Health* **32,** *406 (1942);* Manning and Milam, *South. Med. J.* **36,** *373 (1943)*), biomicroscopic examination of the eyes with a slit lamp was carried out. A study was made of the types of food which served as the chief source of various vitamins, minerals, and calories.

The results of the study are presented in a series of tables in which are given means from each group and the percentages of the entire group which fall into the five categories: (1) those obtaining the full daily allowances recommended by the Food and Nutrition Board; (2) those obtaining three fourths; (3) those obtaining half; (4) those obtaining one fourth; and (5) those obtaining less than one fourth of the recommended daily allowances. Laboratory data on plasma vitamin A, vitamin C, total protein and albumin, and whole blood hemoglobin are given in detail.

Taking only those who consumed less than one fourth of the daily allowance of vitamin C, 40 per cent of the white and 50 per cent of the Negro groups fell into this class but only 5 per cent of the white and 6 per cent of the Negro groups had zero levels of plasma vitamin C. No scurvy was discovered but gingivitis was more frequent among children with low plasma levels than in those with higher levels. This difference did not exist in adults.

Only 5 per cent of the white and 8 per cent of the Negroes had an intake of vitamin A below one quarter of the recommended allowances and only 4 subjects had less than 50 I. U. of vitamin A per 100 ml. of plasma.

Over 50 per cent of all groups ingested less than half the recommended allowance of thiamine and about 75 per cent received less than half the allowances of riboflavin. No clinical evidence of thiamine deficiency was detected by careful examination. The incidence of vascularization of the cornea was 73 per cent in the white subjects and only 17 per cent in the Negroes, though the riboflavin intake had been higher in the white subjects.

Of the Negro subjects, 42 per cent ingested less than half the recommended allowance of calcium while only 30 per cent of the whites were below this level. About one fourth of the Negroes ingested less than half the allowance of iron while only 15 per cent of the white subjects were below this level. Less than half the allowance of protein was ingested by 8 per cent of the white adults and by 13 per cent of the Negro adults, but no plasma proteins were found below 6 g. per 100 ml.

Mean levels of hemoglobin were 14.6 g. per 100 ml. of blood for the white male adults, 0.5 g. lower for the Negro males, 1.5 g. lower for the white females, and 2 g. lower for the Negro females. Values for children under 12 years of age were lower; there was no difference between the values for boys and girls but the white children had higher levels than the Negro.

The calory intake was consistently below 2000 calories daily—averaging 1950 for white adults and 1750 for Negro adults. Less than 1500 calories, half the recommended allowance, were ingested by 21 per cent of the white adults and 37 per cent of Negro adults. Low intakes were more fre-

quent in women than in men. In spite of this level of intake, 20 per cent of all white and Negro adults were 20 pounds or more overweight, whereas only 14 per cent of the white and 11 per cent of the Negro adults were 20 pounds or more underweight. Among the children, about twice as many were underweight as overweight.

It is at once apparent from the inharmoniousness of the several classes of data that different interpretations are possible. Using the records on their face value, one could justify statements that (1) the intake of nutrients is dangerously low; (2) the standards are unrealistically high; (3) the physical signs studied are largely unrelated to intake of specific nutrients.

It would appear that there is a fundamental fallacy inherent in the short-term nutrition survey in which dietary history or careful records of food intake, physical examinations, biochemical and microbiologic determinations are carried out in so short a span. It is known that such data, gathered simultaneously, contain inconsistencies. The clinical signs which are diagnostic of deficiency disease usually result from protracted deficiencies; the biochemical status at an instant in time is a composite of underlying tissue stores or deficits and the balance between recent losses and recent accruals from intake; and the dietary story is accurate only for the particular time when records are kept. This picture is brought into focus by the use of tables of food values which are rarely as precise as the data on intake. Exact information on the nutritional status, and more importantly its trend of improvement, deterioration, or constancy, is no more available from such a survey than is an understanding of the pathologic physiology of a complex disease from a single microscopic slide. Until it is possible to extend the nutrition survey in time so that it encompasses the significant period during which the humoral phase of deficiency becomes translated into the diagnostic sign it will be a tool of limited value. It is to be hoped that in the future protracted studies will be carried out which, by following the slow stages of development, will give a more active picture of deficiency disease than the single phase static picture now available.

A PARA-AMINOBENZOIC ACID PEPTIDE AS GROWTH FACTOR FOR MICROORGANISMS

An idea proposed by Woods and Fildes (*Chem. Ind. (London)* **18,** *133 (1940)*) that the bacteriostatic effect of sulfonamides might be explained as an antagonism to the action of para-aminobenzoic acid has given rise to a large number of papers. Much interest has been shown concerning the fact that growth inhibition due to sulfonamides can be reversed by the addition of para-aminobenzoic acid to the culture media.

As a result of this observation, a number of investigators have recently been interested in testing derivatives of para-aminobenzoic acid either for inhibitory action (antivitamin action) or for growth stimulating action. Particularly the latter problem has attracted many investigators since it was known that para-aminobenzoic acid occurs in a bound form in yeast as well as in liver (MacLeod, *J. Exp. Med.* **72,** *217 (1940)*) and is liberated after acid hydrolysis.

Auhagen (*Z. physiol. Chem.* **277,** *197 (1943)*) in a search for such para-aminobenzoic acid compounds has synthesized a number of peptides of para-aminobenzoic acid. The glycine, glycyl-glycine, and 1-and d-leucine para-aminobenzoic acid compounds were inactive. However, para-aminobenzoyl-l-glutamic acid unexpectedly turned out to be highly active. The corresponding para-aminobenzoyl-l-aspartic acid was completely inactive as was para-

aminobenzoyl-d-glutamic acid. It is rather essential for the discussion of subsequent studies of the same problem to describe in some detail the test employed by Auhagen. The test organism used was Streptobacterium plantarum which will not grow in the absence of para-aminobenzoic acid. No efforts were made to remove completely traces of para-aminobenzoic acid which might naturally occur in the medium or in the glucose used. These were neutralized by the addition of small amounts of sulfonamides. Thus, 20 micrograms of sulfanilamide per liter of test medium were sufficient to counteract any impurities of para-aminobenzoic acid. Addition of a few millimicrograms of para-aminobenzoic acid to such a system gave rise to marked growth. The para-aminobenzoyl-l-glutamic acid was found to be even more active than para-aminobenzoic acid. Thus, 0.3 millimicrograms per milliliter of this compound gave rise to a growth response corresponding to about 3 millimicrograms per milliliter of para-aminobenzoic acid. Therefore, para-aminobenzoyl-l-glutamic acid is about ten times more active as a growth factor for Streptobacterium plantarum than para-aminobenzoic acid. Mixtures of l- and d-glutamic acid peptides showed a strict proportionality between the percentage of the l-component present and growth response. Thus, the fivefold concentration of a mixture of 20 per cent of the l-form + 80 per cent of the d-form, and the twenty-five fold concentration of a mixture of 4 per cent of the l-form + 96 per cent of the d-form were required in order to obtain the same growth response as that given by a certain amount of the pure l-form.

Williams (*J. Biol. Chem.* **156,** *85 (1944)*) has compared the growth response brought about by para-aminobenzoic acid and by para-aminobenzoyl-l-glutamic acid using various strains of microorganisms. An endeavor was made to prepare media completely free of para-aminobenzoic acid. The sulfonamide and the para-aminobenzoic acid compound were then titrated against each other, i.e., the smallest amount of a para-aminobenzoic acid compound which was able to give rise to growth in the presence of a certain amount of a sulfonamide compound was determined.

Williams found that in most cases para-aminobenzoyl-l-glutamic acid was almost inactive as a sulfonamide antagonist. Thus, the effect of para-aminobenzoic acid was 6000 to 8000 times greater as an antagonist toward sulfonamides than para-aminobenzoyl-l-glutamic acid when Staphylococcus pyogenes or Escherichia coli was used as the test organism. Only with Lactobacillus arabinosus as test organism in a semi-synthetic medium did para-aminobenzoyl-l-glutamic acid show any appreciable action. In this test the peptide showed one-twentieth of the effect of that of the free para-aminobenzoic acid.

The test using Lactobacillus arabinosus in a semisynthetic medium comes nearer to Auhagen's test system than any of the other test systems used, and therefore it may not be without significance that only in this system did para-aminobenzoyl-l-glutamic acid show any significant activity. Unfortunately, Streptobacterium plantarum is not available in this country, so it has not been possible to repeat Auhagen's experiments. In any case, the action of para-aminobenzoyl-l-glutamic acid seems to be limited to certain strains of bacteria.

The interest in para-aminobenzoyl-l-glutamic acid has been further stimulated by the isolation from yeast of a para-aminobenzoic acid peptide which contains only glutamic acid (Ratner, Blanchard, Coburn, and Green, *J. Biol. Chem.* **155,** *689 (1944)*), there being 10 molecules of glutamic acid to each molecule of the compound. This para-aminobenzoic acid peptide failed to show any activity as a sulfonamide antagonist. However, this may be ascribed to its molecular complexity which might prevent its passage into the bacterial cell.

Likewise, it is possible that the findings

of Williams can be ascribed to the varying ability of different strains of bacteria to permit passage of the para-aminobenzoyl-l-glutamic acid, although it must be admitted that this is speculation and that other mechanisms are possibilities.

THE ABSORPTION OF FERROUS AND FERRIC RADIOACTIVE IRON

One of the basic therapeutic principles in medicine is the value of iron in treating anemia of the hypochromic microcytic type. There is ample indication that man absorbs ferrous iron better than ferric iron, and that it is much more efficient in the treatment of iron deficiency anemia (*Nutrition Reviews* **1,** *154 (1943)*). Experimental animals seem to behave in a different manner, and reports from the medical literature disagree as to whether ferric or ferrous salts are better absorbed, or if there is any difference between the two types. This means that experimental animals absorb salts of iron in a manner different from the normal or iron deficient human subject, or that the experimental approach does not give valid results. Hahn and associates have developed a satisfactory technic for measuring iron absorption by use of radioactive isotopes (Hahn, Bale, Lawrence, and Whipple, *J. Exp. Med.* **69,** *739 (1939)*). This method was employed by Moore, Dubach, Minnich, and Roberts (*J. Clin. Invest.* **23,** *755 (1944)*) in a comprehensive study of the absorption of different iron salts in normal and iron deficient dogs and normal and iron deficient human subjects.

Six normal dogs were given 1 mg. of ferrous or ferric radioactive iron per kilogram of body weight and the hemoglobin synthesized with this iron was measured. Nine comparisons of the two forms of iron were made: more ferrous than ferric iron was absorbed six times while approximately the same amount of each was absorbed in the remaining three experiments. The behavior in 2 of the 3 dogs tested twice was not consistent both times. Four dogs rendered anemic by systematic bleedings were given the two types of iron. They absorbed considerably more iron than did normal dogs and there was no preferential selection of one type over the other.

Eight normal adult male subjects with normal red cell, hemoglobin, and hematocrit values were given 1, 2, or 4 mg. of ferrous and ferric salts of radioactive iron per kilogram of body weight after a twelve hour fast. In each case more ferrous than ferric iron was absorbed, the ratios ranging from 1.5 to 10. Similar studies on 5 patients with severe hypochromic microcytic anemia, treated in a similar fashion, absorbed from two to fifteen times as much ferrous as ferric iron. Just as in the animal experiments the human subjects with iron deficiency anemia absorbed a considerably larger percentage of small doses of iron than of large doses, and the iron was assimilated more completely by the anemic females than by the normal males. These observations on human subjects are in complete agreement with those of other clinical investigators.

This study is especially valuable because one group of workers has used identical methods, technics, and apparatus on both human and animal subjects in an effort to resolve the conflicting testimony. Human subjects absorbed ferrous iron more efficiently than ferric iron; this was true in 6 of 13 experiments in dogs while in the remaining 7 there was no preferential absorption of iron with either valence. Moore and associates (*loc. cit.*) suggest three possible reasons for the greater absorption of bivalent iron by the human subject. First, it is possible that only ferrous iron can be absorbed, and all trivalent iron would have to be reduced before it became available for absorption. If reduction is slow or incomplete less ferric iron would ultimately be

absorbed. Secondly, there may be a selective or preferential absorption of ferrous iron, and a slower and less complete absorption of ferric iron. Finally, ferric iron may be rendered less accessible because it readily forms complex compounds of low solubility within the alimentary canal. It is presumed that the dog, in some instances, is able to assimilate ferric iron as well as ferrous iron because these factors are not operative.

This type of investigation is eminently desirable because it emphasizes a point which must never be lost sight of by the experimenter—namely, the inherent differences among species of animals as regards many basic biologic functions and characteristics. Advances in clinical medicine and nutrition are made when a specific problem, perceived at the bedside, is made the subject of a properly controlled laboratory experiment on animals and the results or suggestions arising from the laboratory investigation are brought back into the clinic for final evaluation on the normal or sick human subject.

The fact that evidence from investigations on various experimental animals and human subjects is at odds does not mean that this or that evidence is false or that technics are in error, but often indicates merely the axiomatic truth that biologic reactions vary with species. When this is demonstrated by an approach which includes more than one species, practical advances in medical knowledge are made.

CONGENITAL AND INFANTILE BERIBERI

A case of beriberi in a newborn infant reported from Baton Rouge, Louisiana, by Van Gelder and Darby (*J. Pediatrics* **25,** *226 (1944)*), is apparently the first instance of the congenital occurrence of this disease reported from an occidental country. Infantile beriberi is common in the Orient, but even there it has been rarely reported in the newborn although its development in early infancy is presumably conditioned by maternal dietary deficiency during pregnancy. In Japan beriberi was ranked by Ching-Lang (*China Med. J.* **50,** *324 (1936)*) sixth in a list of causes of infant deaths, and in the Philippines in 1928 it was reported that 16,500 infantile deaths from this disease occurring annually represented 28.1 per cent of the total deaths among infants under 1 year of age in that territory (Lopez, *J. Philippine Islands M. A.* **8,** *422 (1928)*). In the United States, cases of thiamine deficiency in infancy have been reported by Haas (*Arch. Pediatrics* **46,** *467 (1929)*), Waring (*Am. J. Dis. Child.* **38,** *52 (1929)*), and Rascoff (*J. Am. Med. Assn.* **120,** *1292 (1942)*).

In the case reported by Van Gelder and Darby, the infant was born to a mother who had limited her diet to apples, bananas, grapes, grapefruit, tomatoes, raw turnips, and approximately 6 slices of white bread, with less than 1 glass of milk daily. She had mild edema of her face and extremities during the last three months of pregnancy, with normal blood pressure and urinalyses. Mild toxemia of pregnancy was suspected. The infant, of normal weight, became cyanotic immediately after birth and subsequently had repeated cyanotic attacks requiring the administration of oxygen. Retraction of the head and convulsive movements developed at thirty hours. At forty-two and fifty hours after birth, 50 mg. doses of thiamine hydrochloride were administered subcutaneously. Twelve hours after the second dose the baby appeared dramatically improved. The color became normal and cyanotic attacks did not recur. The heart rate, initially very rapid, slowed. At 3 months of age opacities of both lenses were observed and it was thought probable that the cataracts were present at birth. While the authors ascribed this occurrence of cataracts to thiamine deficiency, it should be

noted that the avitaminosis was multiple in both mother and infant, and that the mechanism of cataract production is not clear.

The mother's edema disappeared a few days after delivery, concomitantly with her taking a well balanced diet in the hospital, but during the second postpartal week she developed mild cheilosis, suggestive of ariboflavinosis, that persisted ten days. After three weeks the mother was given, for two weeks, a diet limited to the foods she had taken during the last trimester. Dextrose, pyruvate, and lactate blood levels, both fasting and following the ingestion of dextrose, and urinary thiamine assays, indicated a state of thiamine-deficiency in the mother. Van Gelder and Darby point out that thiamine deficiency should be suspected when mild symptoms of toxemia of pregnancy develop and recommend the parenteral administration of thiamine in such cases. It is to be emphasized that although the mother had "subclinical" beriberi the baby was born with severe, almost fatal manifestations. With increasing appreciation of the significance of subclinical states of vitamin deficiency this case report should direct attention to the fact that mild vitamin deficiencies probably exist more commonly, in both mothers and infants, than is generally recognized. This report on congenital beriberi resulting from a faulty maternal diet, associated with congenital cataract, also lends emphasis to the practical importance of the recently reviewed investigations (*Nutrition Reviews* **2,** *297* (1944)) on maternal diet in relation to congenital malformations.

VITAMIN DEFICIENCIES IN THE MALAY STATES

In 1939 and 1940 the Institute for Medical Research in Kuala Lumpur, Federated Malay States, undertook a nutritional survey of that area. Fasal (*Arch. Dermat. Syph.* **50,** *160* (*1944*)) has presented a brief account of his observations.

The study included 1482 Malays who were examined in their rural schools and 3657 Tamil (Indian) children who were seen on the rubber plantations where their parents worked. The subjects ranged in age up to 18 years. Although the study was apparently rather extensive, many of the data were not available to the author and his report is confined to three types of changes that were observed.

One was a hyperkeratotic, follicular skin lesion such as that described by various workers, notably Frazier and Hu (*Arch. Dermat. Syph.* **33,** *825* (*1936*)), as characteristic of vitamin A deficiency. Although these skin changes were found in only 2 per cent of the Malay children they were seen in 25.6 per cent of the Tamil children. The author noted that external irritation, as by pressure, was to a certain extent responsible for the localization of the early lesions. He states that in the majority of the cases intake of vitamin A led to rapid regression of the lesions. Vitamin A was supplied by feeding red palm oil, a substance which furnishes 1000 I.U. of vitamin A per milliliter. It is stated that the results with red palm oil were excellent, not only when given by mouth but also when applied locally. Coconut oil, which contains no vitamin A or carotene, was applied locally in controls and gave negative results. In a few rare instances the lesions did not improve or improved only slightly after intake or application of vitamin A.

Bitot's spots were found in 2.9 per cent of the Malay children and in 8.0 per cent of the Tamils. By Bitot spots the author means circumscript areas of xerosis of the conjunctiva situated laterally to the cornea and sometimes forming well defined white spots. The shape of these spots was circular, oval, or triangular with the apex towards the canthus of the eye. An excellent

illustration of one of these is published. The author noted a decided regressive change in these eye lesions after vitamin A medication.

A third type of lesion observed was an angular stomatitis which was found in 4.7 per cent of the Malays and in 22.2 per cent of the Tamils. Occasionally glossitis and cheilitis were present concurrently. It is stated that these lesions subsided when riboflavin alone or the whole vitamin B-complex was administered.

Relatively few Chinese were examined. Among these, many cases of beriberi were observed but no cutaneous lesions except edema were seen. Not a single case of phrynoderma or Bitot spots, and only rare instances of angular stomatitis were found in the Chinese.

These observations corresponded well with the diets of the several groups of subjects. Except for the fact that the rice they ate was polished, the diet of the Chinese was far better balanced than that of the Malays and was superior especially when compared with that of the Indians. The Chinese ate vegetables, including bean sprouts, and pork, fish, soybean cake, and soybean curd. The diet of the Tamils living on the estates consisted mainly of rice prepared with curry and other condiments, few green vegetables, little meat and eggs, and no milk. The only fruit eaten was the banana. This diet was considered to be lacking in proteins, fat, vitamin A, calcium, and iron. The diet of the Malays in the rural areas consisted also mainly of rice with curry, little meat, few eggs, and no milk, but a large amount of green vegetables, fruits, and fresh or dried fish was eaten in addition.

A decided improvement in the general condition of the subjects was observed after they had consumed a special ration which the author induced the plantation managers to feed to their workers. This consisted of soybeans, Indian lentils, rice flour, and skim milk powder and furnished 555 calories with 34 g. of protein, 0.4 g. of calcium, about 12,000 I.U. of vitamin A, and a good supply of the B-complex.

The occupation of the Malay States by the Japanese brought this interesting study to an abrupt conclusion and made it impossible for the author to gain access to his records.

TOPICAL APPLICATION OF VITAMINS TO WOUNDS

Since vitamins have a general growth promoting property and since dietary measures tend to improve the condition of the subject as a whole and in turn increase the rate of healing of wounds, Williams and Bissell (*Arch. Surgery* **49**, *225* (*1944*)) undertook to study the effect of the topical application of vitamins and of some other chemicals on the healing of wounds in normal rats.

The animals used were of the Wistar strain. They had been fed fox chow, carrots, and celery leaves for several weeks before the beginning of the experiment. The majority had also been given cod liver oil and brewers' yeast occasionally but they received neither of these substances for about one month before the study was started. More than 100 animals were used. Wounds of several different types were made on the back. For example, in one experiment an elliptic area of skin was excised, the incision extending into but not through the corium. In another group the lesion produced extended into the subcutaneous layer, while in a third group all of the tissue outside the thoracic cage was excised. The water soluble vitamins used were suspended in sesame oil in order to delay their absorption and thereby prolong their exposure to the margins of the wounds. The substance to be tested was placed directly on the wound and the latter was then covered with a sterile rubberized silk. Care was taken to

house the animals singly where necessary in order to avoid the ingestion of the applied material by other animals. Vitamins or other substances were applied daily and at the same time the degree of healing of the wounds was carefully noted. Biopsies were made from time to time. The substances tested included vitamins A, C, D, and E, thiamine chloride, nicotinic acid, riboflavin, calcium pantothenate, pyridoxine, biotin, hydrosulphosol, biodyne, urea-sulfathiazole ointment, amino acids, adenosine, liver extract, cod liver oil, a vitamin mixture, and sesame oil.

The results in this painstaking piece of work were entirely negative. No definite benefit was derived from the use of any of these substances.

INTERPRETATION OF SELECTIVE SERVICE STATISTICS IN TERMS OF NUTRITION

It has been implied that a high percentage of men rejected by Selective Service has been due largely to malnutrition (*Bull. National Research Council no.* **109,** *38 (1943)*). One widely circulated legislative report states that "one-third of the draftees found unfit for service during 1941 were rejected directly or indirectly because of nutritional deficiencies" ("The Nutrition Front," *New York State Joint Legislative Committee on Nutrition, Legislative Document no.* **64,** *Albany (1943)*). An examination of two of the reports on the findings of Selective Service examinations leads one to question that objective evidence exists upon which to base such conclusions.

Thus, Rowntree, McGill, and Edwards (*J. Am. Med. Assn.* **123,** *181 (1943)*) have listed the causes of rejection of 18 and 19 year old registrants according to defects. An examination of their data reveals few defects which may with certainty be attributed directly or indirectly to malnutrition. For example, musculoskeletal defects which accounted for 22.7 rejections per 1000 examinees, were distributed among the following groups: amputations, osteomyelitis, ankylosis, arthritis, atrophy, injury, spinal malformations, and congenital defects. In discussing underweight the authors state that "no data were recorded on the examination form to show how frequently this or any other defect could be presumed to be the result of malnutrition." This conclusion is in marked contrast to the testimony of Scott before the New York Legislative Committee on Nutrition (*loc. cit., p. 186*) to the effect that "of the first million selectees called, 188,000 or 20.9 per cent were rejected for dental defects, 61,000 or 6.8 per cent were rejected for musculoskeletal defects, 36,000 or 4.0 per cent were rejected for defects of the feet. Now all of these defects may be directly related to the minerals calcium and phosphorus."

Perrott (*Milbank Memorial Fund Quart.* **22,** *358 (1944)*) has listed the ten leading causes of rejection by Selective Service examiners as of February 1, 1944, as follows: mental disease, syphilis, musculoskeletal defects, cardiovascular, hernia, neurologic, eyes, ears, mental deficiency, and tuberculosis. Again, one would be hard pressed to defend on the available evidence the thesis that any one of these causes was primarily of nutritional origin. The ten leading causes of rejection in World War I are listed by comparison as: musculoskeletal, cardiovascular, eyes, tuberculosis, underweight, feet, hernia, ears, mental deficiency, mental and neurologic disease. Although comparison is rendered difficult because of differences in age composition, examination standards, and examining technics, one cannot conclude that these data indicate that great deterioration of health has resulted in the period between the wars as a result of poor diet. Obviously, a more conservative interpreta-

tion than some enthusiastic nutritionists have shown of the role of malnutrition as a causative agent in such physical disability is called for until more objective evidence upon which to base their claims is forthcoming.

An indication of the types of relationships which may ultimately direct us to such evidence is seen in the paper of Ciocco, Klein, and Palmer (*Pub. Health Reports* **56**, *2365 (1941)*). This paper compares the physical status of selectees who were accepted for military service and those who were not accepted because of physical or mental defects with the records of the physical status of the same youths some fifteen years previously. The earlier school examinations had been made in the white schools of Hagerstown, Maryland, by the United States Public Health Service. The findings indicate that underweight, poor nutritional status, the number of filled, missing, or carious teeth, and bad posture were much more frequent among children who fifteen years later were rejected for military service than among the children who as youths were accepted for military duty. Thus, of the 132 children who at the time of the school examination, were judged as having good posture, 60.5 per cent were fifteen years later placed in Class 1-A. By contrast, of the 168 selectees who had been judged as children as exhibiting fair or poor posture only 38.3 per cent were placed in Class 1-A. The authors conclude in part that "the results of the present study suggest also that growth as measured by weight, posture, and the physician's estimate of the state of nutrition—all traits that are frequently examined to determine the health status of the child—are useful as crude predictive indexes of adult physical development." These correlations are of particular significance because of the tendency in recent years to discount the value of weight measurements, gross clinical evaluation of "nutritional state," and evaluation of posture in favor of newer diagnostic procedures.

The great variety of environmental and constitutional factors which contribute to the attributes here discussed prevents one from assigning a predominate role to any single factor such as diet. For example, it was found that the rejected group had a smaller percentage of their carious teeth filled in childhood than did that group which later met Selective Service physical standards. This indicates that the better group had more adequate dental care at the time of the examination. It would be logical to attribute the adult state of the teeth largely to this factor. Such correlations as Ciocco *et al.* have obtained do not necessarily signify causation; but they do indicate the direction toward which further explorations might profitably be guided in efforts to seek out relationships between dietary habits and immediate and future nutritional status.

MASS CONTROL OF DENTAL CARIES

One of the most interesting studies to be undertaken in the field of public health is the attempt to control dental caries (decay) in certain communities by the addition of fluorides to public supplies of drinking water. One study area for such a project is the city of Newburgh, New York. It is proposed to add sodium fluoride to the Newburgh water supply in sufficient quantities to bring its fluorine content to a concentration just below 1 p.p.m. The nearby city of Kingston, in which the water supply is nearly fluorine free, is to be the control area for this study.

There can be no argument as to the need of improving dental health. Dental decay has been termed the most prevalent disease of civilized man. Few individuals escape its attack and usually not one but many teeth are affected. Control of dental caries through reparative measures appears to be

an impossible task. Dental enamel once injured is incapable of self repair, and carious defects tend to accumulate at a rapid rate. American dental practitioners, now numbering approximately 70,000, spend the major portion of their time in repairing damages attributable directly or indirectly to dental caries, yet a majority of the people receive either no dental service at all or merely extractions. The chief hope of solving the dental problem of the population as a whole lies in drastically reducing the incidence and extent of tooth decay. Fluorination of drinking water may offer a unique opportunity for progress in this direction.

The attempt at local mass control of dental caries by this method has a firm foundation in scientific fact. In the past decade, convincing data have been secured from epidemiologic surveys, from chemical studies, and from experiments with animals to attest to the importance of fluorine in dental health. It has been shown that the incidence of dental decay varies inversely with the concentration of fluorine in the water supply; that the enamel of sound teeth has a higher fluorine content than the enamel of carious teeth; and that under special circumstances induced caries in rats may be prevented to a significant degree by additions of sodium fluoride or calcium fluoride to the animals' food or water.

Two important points have been brought out by epidemiologic studies; first, that it is almost certainly the fluorine in domestic waters which accounts for the low incidence of dental caries in certain localities and, second, that certain levels of fluoride, which are too low to cause disfiguring mottled enamel, appear to be capable of reducing the amount of dental decay. It has long been known that despite their defective structure, teeth with mottling of the enamel are less susceptible to decay than teeth not so affected. The discovery, in 1931, that fluorine is the causative factor of mottled enamel gave impetus to the study of this agent in the prevention of dental caries. In 1938, Dean (*Pub. Health Reports* **53,** *1443 (1938)*) presented data indicating a comparatively high percentage of caries-free children in communities with a high concentration of fluorine in the drinking water and noted that the relative freedom from caries was present whether or not the teeth showed macroscopic evidence of mottled enamel. Later studies (*Nutrition Reviews* **1,** *182 (1943)*) showed that there is an inverse relationship between the prevalence of dental caries and the fluoride content of the water supply but that there is no correlation between the caries experience rate and such factors as hours of sunshine, the economic level of the community, or the hardness of the drinking water. They showed, also, that low dental caries is associated with the continuous use of domestic water whose fluorine content is as low as about 1 p.p.m. This concentration under the conditions prevailing in the localities studied produces only sporadic instances of the mildest forms of dental fluorosis, of no esthetic significance.

After consideration of the various aspects of the problem, Arnold (*J. Am. Dental Assn.* **30,** *499 (1943)*) proposed the controlled addition of fluorides to public water supplies in selected communities. He says: "The results of epidemiologic, chemical and experimental studies suggest that the addition of small amounts of fluoride, not to exceed 1 part per million, to fluoride-free public water supplies may be a practical and efficient method of markedly inhibiting dental caries in large group populations." From epidemiologic studies, Arnold calculated that the following benefits might be expected in children born after the change in the fluoride content of the drinking water: (1) about six times as many children showing no dental caries experience (caries free); (2) about a 60 per cent lower dental caries experience rate; (3) about a 75 per cent decrease in the first permanent molar loss; and (4) approximately 95 per cent less caries in the proximal surfaces of the four upper

incisors. Arnold considered carefully two objections which might be raised to the addition of fluorides to public water supplies, namely the danger of cumulative systemic toxic effects, and the danger of dental fluorosis (mottled enamel). He feels that the danger of cumulative toxic effects is rather remote since in some areas in the United States people have been using water with six to eight times the proposed concentration of fluorine for as long as forty years with no reported pathologic effects, other than on the teeth. In regard to dental fluorosis, Arnold pointed out that in communities using a water supply which contains about 1 p.p.m. of fluorine the mottled enamel encountered is of a mild variety. In one such community (Aurora, Ill.), of 16,448 permanent teeth examined, only 57 incisor teeth were diagnosed as positive. For no tooth was there a diagnosis of "moderate" (brown stain) or "severe" (pitting) fluorosis. The mild fluorosis occasionally encountered was in no way disfiguring.

Two reports are of interest in providing data which suggest that continued intakes of fluorine up to 5 or 6 p.p.m. in drinking water are not harmful. McClure (*Pub. Health Reports* **59,** *1543 (1944)*) studied the relation between fluorine ingestion (from drinking waters) and the height, body weight, and bone fracture experience of selected groups of 1458 high school boys and 2529 young adult men taking the physical examination at induction centers. The subjects were from areas in which the water borne fluoride content ranged from 0.0 to 6.0 p.p.m. No relation was found between fluoride intake and incidence of bone fractures nor was there any relation between height-weight figures and fluoride intake.

In the second report McClure and Kinser (*Pub. Health Reports* **59,** *1575 (1944)*) studied the relation between the concentration of fluorine in the water supply and that in the urine of subjects drinking the water. Fluorine analyses were done on urine samples from over 1900 men and boys from areas in which the drinking water varied from 0.0 to 5.8 p.p.m. fluorine. These studies show that where domestic waters are free of fluorine, the urinary fluorine content is low, 0.3 to 0.5 p.p.m. When the domestic waters contain as little as 0.5 p.p.m., detectable increases in urinary fluorine are observed, and further increase in the fluorine content of the waters produces proportional increases in urinary fluorine. These results show a remarkable ability of the body to handle water borne fluorides in the concentrations encountered in these studies, which concentrations are considerably higher than required to give protection from caries. They also indicate that the food fluorine intake is rather constant regardless of the locality, and amounts to about 0.3 to 0.5 mg. daily. It would appear, therefore, that with respect to fluorine the water borne fluorides are of first importance in maintenance of dental health.

It has been suggested that a number of communities add fluoride to their water supplies under controlled conditions. The Newburgh-Kingston demonstration appears to be thoughtfully planned and is to be carefully controlled. Newburgh, a city of approximately 30,000 population, located on the Hudson River, already has about 0.12 p.p.m. of fluorine in its domestic water. Approximately 0.8 p.p.m. is to be added in the form of sodium fluoride. Kingston, a city of similar size with comparable water supply, population group, climatic conditions, economic status, and source of common food supply, is to serve as the control area. The city water of Kingston is now, and is to remain, essentially fluorine free. All 5 to 14 year old children in the schools of Newburgh and Kingston are to have dental examinations annually. A representative group of children in the study area will have complete physical examinations periodically. Physical examinations at regular intervals will also be made on a group of adults.

We must distinguish carefully between

controlled studies of this type and the indiscriminate addition of fluorides to all domestic waters of low fluorine content. It is certain that conservative opinion would condemn the general fluorination of drinking water at this time. After considering the benefits which might result from fluorination of water supplies, Dean (*J. Am. Water Works Assn.* **35,** *1161 (1943)*) cautions: "Much investigative work, however, is necessary before serious thought can be given to a recommendation for its general application. To determine the safety threshold with regard to possible effects other than on the teeth, carefully controlled studies must be made of populations who have used fluoride waters of relatively high concentration over a number of years."

As these momentous studies in the Newburgh-Kingston and other areas get under way we should not lose sight of the fact that there are most certainly factors other than the ingestion of proper amounts of fluorides which are important to dental health even though we cannot as yet identify them with certainty. It seems that the influence of the nature of the carbohydrate and a number of other factors in the diet (e.g. protein quality, calcium, and vitamins A, C, and D) might well be reinvestigated under carefully controlled or known conditions of fluorine intake.

THE AMINO ACIDS OF HUMAN MILK

The relative value of human and cow's milk for infant feeding is a matter of perennial interest. A paper by Williamson (*J. Biol. Chem.* **156,** *47 (1944)*) is of importance in this connection as it adds to our knowledge of the amino acids contained in the proteins of human milk. Previous work by Plimmer and Lowndes (*Biochem. J.* **31,** *1751 (1937)*); *Compt. rend. trav. lab. Carlsberg* **22,** *434 (1938)* (see also *Chem. Abstr.* **32,** *7950 (1938)*), Beach and co-workers (*J. Biol. Chem.* **139,** *57 (1941)*), and by Beach, Bernstein, and Macy (*J. Pediatrics* **19,** *190 (1941)*) on human casein and lactalbumin had placed on record figures for the percentages of seven of the amino acids in these proteins. These included only five of the ten amino acids recognized as essential by Rose (*Science* **86,** *298 (1937)*), namely tryptophane, methionine, lysine, histidine, and arginine. In addition values for cystine and tyrosine were reported by both groups of workers.

Williamson collected and pooled specimens of human milk, keeping them frozen until a sufficient amount was on hand for analysis. From 5 gallons of the composite human skim milk the casein and lactalbumin were separated. They were analyzed for moisture and for ash content, and all amino acid analyses were calculated on a moisture-free, ash-free basis. For purposes of comparison the casein and lactalbumin of cow's milk were also prepared by similar methods. Each of the four proteins was then analyzed for each of eighteen amino acids, including all ten of the essential amino acids. The remaining amino acids determined were cystine, serine, aspartic and glutamic acids, proline, glycine, alanine, and tyrosine.

From the data on the human casein and lactalbumin Williamson has calculated the amount of each of the amino acids in human milk on the basis of average figures for its protein content—0.5 per cent casein and 1.0 per cent lactalbumin. The figures vary from no glycine at all to 228 mg. per cent isoleucine and 230 mg. per cent glutamic acid in the human milk. They were compared with the similarly calculated figures for cow's milk diluted 1:1 with water, a solution containing 1.4 per cent casein and 0.25 per cent lactalbumin, because this is the dilution of cow's milk which is most commonly employed in infant feeding. The comparison

shows that tryptophane is the only essential amino acid which occurs in lower concentration in the diluted cow's milk than in human milk, and that for thirteen of the amino acids measured the concentrations in the diluted cow's milk and in human milk are remarkably close. The remaining five amino acids together with the ratio of their concentration in diluted cow's milk to that in human milk are: proline 1.55; glutamic acid 1.48; aspartic acid 0.72; cystine 0.34; and methionine 1.72. When the total sulfur-containing amino acid content is expressed in millimoles, the figures for diluted cow's milk and for human milk are not significantly different.

The practice of diluting cow's milk with an equal volume of water for infant feeding is based on the fact that the total protein content of cow's milk is on the average twice as great as that of human milk. It is therefore of much interest to learn from the work of Williamson that the ratio of the amino acid content of cow's milk to that of human milk is so close to 2:1 for each of the essential amino acids except tryptophane and methionine, and that the total sulfur-containing amino acids occur in just this ratio.

SEVERE ANEMIA OF PREGNANCY IN INDIA

A report by Upadhyay (*Indian Med. Gaz.* **79,** *193* (*1944*)) draws attention again to the problem of severe anemia of pregnancy encountered in India, and brings evidence to show that malnutrition plays an important part in the etiology of such anemia. Healthy women of this region had been studied previously by Prasad and Chowdhury (*Ibid.* **78,** *429* (*1943*)), who made observations on the hemoglobin levels of 71 women "showing no abnormal signs or symptoms and looking healthy." The average hemoglobin content was 12.5 g. per 100 ml. of blood.

Upadhyay first studied the blood picture of 51 apparently normal pregnant women, and 86 apparently normal nonpregnant women of childbearing age. His figures for hemoglobin are somewhat higher than those of Prasad and Chowdhury, and he also determined the total red cells, reticulocytes, total leucocytes, eosinophils, and the plasma protein concentration. His observations support the concept of a "serous plethora" or "physiological anemia" of pregnancy. The mean hemoglobin levels were 13.5 g. per cent for the nonpregnant and 12.5 g. per cent for the pregnant women. Red cells numbered 4.7 and 4.1 million per cubic millimeter respectively; plasma proteins were 7.1 and 6.8 g. per cent respectively. The total leucocyte count however was increased on the average from 6300 per cubic millimeter in the nonpregnant to 11,300 in the pregnant woman.

The anemic pregnant women were almost all clinical cases of grave anemia. Among the 58 anemic pregnant women examined, only 3 were of the microcytic type, while 44 were macrocytic. Twenty-three were from 15 to 20 years old, 16 were 21 to 25, 12 were from 26 to 30, and 7 were over 30 years old.

The hemoglobin level in these patients varied from 1.6 to 6.5 g. per 100 ml. of blood, and was below 4 g. per cent in 33 of them. The total red cell count varied from 0.5 to 2.5 millions per cubic millimeter; in 26 women it was less than 1 million, and in 25 others it was between 1.0 and 2.0 millions. The plasma protein concentration varied from 0.7 to 5.0 g. per 100 ml. of plasma and was below 4.0 g. in 47 of the patients. The clinical picture of these women shows generalized edema, weakness, dyspnea, and marked anorexia, which doubtless accounts for the author's observation that "the one outstanding feature of the pregnant anemic woman is the calm composure with which she reports herself in the hospital (if not brought in an unconscious state by the maternity service)."

Upadhyay has adopted a nutritional therapy for these grave cases, giving whey (since milk protein is easily utilized) later fortified by egg albumen. Whole liver is also used, in the form of liver juice or grilled cubes of liver "as digestion permits." He has found that the patients do well when able to swallow and digest protein; if the appetite returns and the patient eats well without diarrhea occurring, recovery may be rapid. When the patient improves by any method of treatment, the plasma protein content immediately rises. It seems likely therefore that dietary deficiencies, and particularly protein deficiency, are of first importance in the etiology of this anemia.

This view receives support from the statistical studies reported. Neither the age range nor the parity of the patients appears to give useful information about the etiology. The proportion of cases seen among Hindus and among Moslems is stated to be similar to the proportion of these two groups in the population, as is to be expected, because among the poorer classes the manner of life is similar in different communities. When the patients were classified according to the diet they had previously eaten, the diets were separated into six classes of increasing quality. The number of cases of anemia found in the six diet classes were 32, 15, 6, 5, 0, and 0 respectively, indicating a close correlation between poor diet and occurrence of the anemia. It was also noted that no case of anemia occurred in women who had been taking at least 1 pint of milk daily. Meat, fish, and eggs were absent from the four poorest classes of diet, and occurred only occasionally in the fifth class. This fifth class of diet, on which no anemia was observed, consisted of rice, wheat flour, fruits, 1 pint of milk daily, and occasionally butter, fish, meat, or eggs.

The conclusion appears to be justified that much of the severe anemia of pregnancy seen in India is due to malnutrition, particularly to lack of protein, occurring as a result of poverty. The anemia is of such severity as is rarely if ever encountered in this country. Unfortunately the author is unable to give any precise account of the incidence of such anemia in Bihar, since only a fraction of the population makes use of the clinics and it is impossible to estimate how much anemia goes unrecorded. However, he remarks that it is an outstanding problem in that region.

NICOTINIC ACID PRECURSORS IN FOODS

It was demonstrated by Kodicek (*Biochem. J.* **34,** *712 (1940)*) that analysis of animal tissues for nicotinic acid by the König reaction gave the same results when the tissues were extracted with hot water and subsequently hydrolyzed by alkali, as when the tissues were extracted by hot alkaline solutions. In cereals however, the apparent nicotinic acid content was much smaller when water extraction was used than when alkaline extraction was used. Kodicek concluded that the apparent nicotinic acid extracted by water was the only true nicotinic acid, and that the large increment in apparent nicotinic acid obtained by alkali extraction did not represent true nicotinic acid, but was due to some other chromogen.

That this is probably correct was shown by Dann and Handler (*J. Biol. Chem.* **140,** *201 (1941)*) who found that in using the König reaction any modification which does not involve almost complete decolorization of the extracts used for the color reaction led to false high values for nicotinic acid. Since Kodicek's alkali extracts would be much more deeply pigmented than his hydrolyzed aqueous extracts of cereals, and since he did not decolorize his extracts, no definite conclusion can be drawn from his experiments.

Different methods of extracting nicotinic acid from cereals were later studied by Andrews, Boyd, and Gortner (*Ind. Eng. Chem.* (*Anal. Ed.*) **14,** *663* (*1942*)), who determined the nicotinic acid content of their extracts by the microbiologic assay method of Snell and Wright (*J. Biol. Chem.* **139,** *675* (*1941*)). They were able to show that water and dilute acids extracted less nicotinic acid from wheat flour than did stronger acids or alkalies. Also they found that an autoclaved aqueous suspension of wheat flour contained more than twice as much apparent nicotinic acid after five minutes' treatment at room temperature with 0.25 per cent of sodium hydroxide than before such treatment, thus showing that the aqueous extract itself is changed in some way by dilute alkali. These authors believed that this phenomenon could best be explained by postulating the existence of an inactive or less active precursor of nicotinic acid, which could be hydrolyzed by alkali or by strong acid. If this hypothesis were correct, proof should be obtainable by demonstrating the nutritional availability of the active product of hydrolysis. However, such a proof presents difficulties on account of the large error of the bioassay for nicotinic acid, unless the precursor could be purified from free nicotinic acid. Andrews and his colleagues therefore attempted to separate the precursor from the free nicotinic acid in wheat flour, using differential extraction, adsorption, and dialysis procedures. The proportion of precursor in a mixture of precursor with active nicotinic acid was increased by dialysis, but complete separation was not obtained.

Krehl and Strong (*J. Biol. Chem.* **156,** *1* (*1944*)) have made another attack upon this problem. Again using the microbiologic assay with Lactobacillus arabinosus as test organism, they have extended Andrews' work in detail, and continued the attempt to isolate the precursor. They have measured the amount of the precursor in different milling fractions of wheat, and have determined with greater precision what degree of alkalinity is needed to secure hydrolysis of the precursor. In addition they have found that Vitab (a rice bran concentrate) is a rich source of the precursor, in which it is accompanied by three to four times as much free nicotinic acid. Extraction studies did not reveal a solvent which would extract either the nicotinic acid or its precursor preferentially, and precipitation from a mixture of the two did not seem to be a suitable procedure. Adsorption methods gave more promising results, but were not completely successful. Both the free nicotinic acid and its precursor were adsorbed upon charcoal, from which most of the free acid could then be eluted by a mixture of pyridine and methanol, while most of the precursor remained adsorbed. The precursor adsorbed on the charcoal could not be utilized by the Lactobacillus unless the charcoal was first treated with alkali. By such adsorption methods a charcoal preparation was made which contained thirteen and a half times as much precursor as nicotinic acid, but the precursor could not be eluted from the charcoal without hydrolyzing it. Krehl and Strong conclude that the new evidence establishes that the precursor is a complex molecule hydrolyzable to yield nicotinic acid.

Krehl, Elvehjem, and Strong (*J. Biol. Chem.* **156,** *13* (*1944*)) have also reported experiments designed to test the availability of the nicotinic acid of the precursor molecule to higher animals when it is fed as the charcoal adsorbate. These authors eluted some of the precursor from a charcoal adsorbate with alkali, thereby hydrolyzing it, and fed the hydrolyzed material to 2 blacktongue dogs. The response of the dogs indicated that the hydrolyzed material was indeed nicotinic acid. The charcoal adsorbate itself when fed to a dog suggested that about 40 per cent of the adsorbate was available to the dog. Similar tests with chicks also indicated that the precursor hydrolyzes to give rise to nicotinic acid, but that the

precursor itself is not utilized at all by the chick.

However, it must be recognized that the conclusions drawn from these experiments with both chicks and dogs can be regarded as only tentative. The tests with dogs involved so few animals that the results are only approximate in a quantitative sense, and the chick tests showed evidence of too much uncontrolled variation to be the basis of quantitative calculations.

Krehl, Elvehjem, and Strong also demonstrated that the Lactobacillus is unable to utilize simple esters of nicotinic acid as the dog is able to do, and that several sugar derivatives of nicotinamide are partially or wholly utilized, although the utilization is not increased by treating them with dilute alkali.

It appears from these studies that little doubt can persist that some of the cereal foodstuffs contain, in addition to active nicotinic acid, a precursor which yields nicotinic acid on hydrolysis; but we are still in a position only to hazard guesses about its chemical composition or its availability to animals and man.

DIETARY LIVER DAMAGE

Two hepatic lesions have been described as occurring in experimental animals as a result of dietary deficiencies; one is a cirrhosis, resembling in many particulars Laënnec's cirrhosis in man, the other a massive necrosis, frequently involving a considerable portion of the total parenchymal tissue of the liver, and often accompanied or even replaced by hemorrhage. There has been considerable discussion among workers in the field as to the relationship or lack of relationship of these lesions. One group of investigators holds the point of view that this massive necrosis is one stage of the process leading to dietary liver cirrhosis. Another group believes that this particular cirrhotic process involves the death, at any one time, of a limited number of liver cells and that the massive necrosis represents quite another deficiency disease.

Reports by Himsworth and Glynn (*Clinical Sci.* **5,** *93, 133 (1944); Lancet* **I,** *457 (1944)*) tend to substantiate the differentiation of these conditions. Their differentiation is based on both etiologic and anatomic considerations, and they have compared "post-necrotic scarring and nodular hyperplasia" with cirrhosis. This contribution appears to have been made possible by the unique circumstance that some of their animals lived for weeks or months after the onset of massive hepatic necrosis and thus permitted the study of the reparative process.

In their first paper, Himsworth and Glynn (*Clinical Sci.* **5,** *93 (1944)*) reported on the production of massive necrosis and its sequelae and of cirrhosis. Albino rats of the Wistar strain weighing 120 g. to 150 g. were used. The statement is made that each animal was allowed 8 g. of food daily but as many as 10 animals were kept in a single cage with the result that group food consumption, only, could be determined. The diets employed were of two types, high-fat diets and high-carbohydrate diets. The high-fat diets contained 50 per cent of lard, 1 per cent of cod liver oil, 3 per cent of salt mixture, and supplements of thiamine, riboflavin, pyridoxine, and pantothenic acid. The remainder was made up of carbohydrate and protein. The high-carbohydrate diets contained 5 per cent of arachis oil, 1 per cent of cod liver oil, 3 per cent of salt mixture, the B-vitamin supplements, carbohydrate, and protein. One series of diets contained protein in the form of casein, at levels from 2 per cent to 16 per cent, and corn starch as the source of carbohydrate. Other diets contained yeast which, by analysis, furnished

protein and carbohydrate in the ratio of 44:56.

The incidence of acute hepatic necrosis was highest among groups of rats receiving yeast protein and was high whether the level of this protein in the diet was 7.2 per cent or 14.4 per cent and whether the diet was high in fat or high in carbohydrate. When the protein was casein, the incidence of necrosis was, in general, lower and apparently depended both on the percentage of protein and on the relative amounts of fat and carbohydrate in the diet. From considerations of quantity of food ingested as well as composition, however, these workers concluded that in all of these experiments with casein the absolute intake of protein was the determining factor for liver necrosis; they calculated that the animals which developed the lesions were those which ingested from 200 to 500 mg. of casein per day. This observation was instrumental in bringing the authors to the more general conclusion that the sole dietary factor determining the incidence of necrosis was the protein fraction of the diet. This conclusion proved fruitful for subsequent investigations but it must be admitted that their calculations of casein consumption would have been more convincing had their animals been fed individually rather than by groups, for they state: "The smaller rats were jostled away from the feeding bowl by the bigger animals and consequently may have obtained less food." It appears improbable, however, that this defect vitiates their general conclusions. The authors reported that fatty infiltration of the liver did not favor and actually may have protected against the development of hepatic necrosis. It was not surprising, therefore, that the administration of choline tended to increase rather than reduce the incidence of this lesion.

After the rats were placed on the experimental diets there was a latent period, varying from twenty-eight to over one hundred days, in which they showed no evidence of illness. With the onset of the acute stage of the disease, the animals huddled in a corner of the cage, refused food and, if severely affected, either succumbed or had a period of several weeks of obvious illness. In animals killed within a few hours of becoming severely ill, the whole liver was swollen and maroon in color and there were large areas in which all of the parenchymal cells were dead. A day or two later livers from severely affected animals were uniformly yellow in color and closely resembled, macroscopically and microscopically, human livers with "acute yellow atrophy." In similar animals a week or two later livers no longer appeared swollen. They were flabby and dull yellow in color and on the surface there were sharply demarcated and obliquely sunken brick red areas. These livers resembled human livers with "subacute red atrophy." The occasional animal which survived extreme necrosis for several additional weeks showed a markedly distorted liver with deep and irregular scars alternating with areas covered with projecting nodules of liver tissue. The authors term this condition "nodular hyperplasia" from its resemblance to the sequel of "acute atrophy" in man. Their description of these sequelae of massive necrosis constitutes a distinct contribution to the literature of dietary hepatic injury. It does not appear that other investigators have been able to prevent a rapid and fatal termination of the disease and, consequently, have had no opportunity to observe more than the early stages of the reparative process.

Himsworth and Glynn differentiated diffuse hepatic fibrosis (cirrhosis) from liver necrosis and its sequelae on both etiologic and anatomic grounds and also on the basis of the length of time necessary to produce the lesions. In contrast to the usual latent period on favorable diets of forty to sixty days for necrosis, unequivocal evidence of diffuse fibrosis was not obtained with their animals in less than one hundred fifty days. Furthermore, cirrhosis did not develop in animals ingesting diets in which the dietary

protein was supplied entirely by yeast while such diets favored the appearance of hepatic necrosis. Again, there appeared to be a close association between fatty infiltration of the liver and diffuse hepatic fibrosis; factors which delayed the development of one were found to delay also the development of the other. Choline or casein prevented or delayed the appearance of cirrhosis. The authors' description of the cirrhotic process agrees in general with that of earlier investigators, although it differs in some details. The appearance of reticulin fibers stretching between portal areas was recorded as the earliest microscopic change and no mention was made of the presence of ceroid in the cirrhotic livers or other tissues (see *Nutrition Reviews* **2,** *183* (*1944*)).

In their second report, Himsworth and Glynn (*Clinical Sci.* **5,** *133* (*1944*)) identified methionine as the component of protein which prevented the development of hepatic necrosis. The level used was 20.5 mg. per rat per day. Negative results with cystine were reported but only 2.24 mg. per rat per day were administered, an amount shown by other investigators to be entirely inadequate for the purpose. From a quantitative point of view, it seems certain that methionine rather than cystine is the most effective component of casein, but only because of the much greater amounts of methionine found in this particular protein.

More recently, the same investigators (*Lancet* **I,** *457* (*1944*)) have suggested that "the 'cirrhosis of the liver' found among the poor in the Punjab and in the Rand is in fact the final stage of a trophopathic hepatitis due to a dietary deficiency of protein" and that poisons, infections, or pregnancy may serve as predisposing causes of dietary hepatic necrosis (trophopathic hepatitis) in man.

This series of papers adds to the weight of evidence that massive hepatic necrosis may arise because of a deficiency of sulfur-containing amino acids in the diet, that it is anatomically as well as etiologically distinct from experimental dietary liver cirrhosis, and that the latter may develop as a result of a deficiency of choline.

CHOLINE DEFICIENCY STUDIES IN DOGS

Although the importance of choline in the nutrition of the dog was suggested by the discovery of its lipotropic action on the liver of the depancreatized dog (Best, Ferguson, and Hershey, *J. Physiol.* **79,** *94* (*1933*)), study of its deficiency in normal dogs has awaited the use of purified rations. Such a study has been presented by McKibbin, Thayer, and Stare (*J. Lab. Clin. Med.* **29,** *1109* (*1944*)) in experiments with young puppies.

Several different diets were used but in all but one the basic constituents were sucrose (46.5 to 59.5 per cent), purified casein (SMA) (6 to 10 per cent), and extracted peanut meal (30 per cent). These were supplemented with 7 per cent cottonseed oil, 2 to 3 per cent cod liver oil, 4.0 per cent salts (Phillips and Hart), and 0.5 per cent di-basic potassium phosphate. The dogs also received thiamine chloride, pyridoxine, calcium pantothenate, riboflavin, and nicotinic acid in adequate amounts. In the one exception, 15 per cent peanut flour was used instead of peanut meal. Peanut protein was used because of its low content of methionine. The peanut meal or peanut flour was extracted with alcohol and ether to remove choline and choline containing phosphatides.

Although such a basal diet had given good results in studies on choline deficiency in rats (Hegsted, McKibbin, and Stare, *J. Nutrition* **27,** *149* (*1944*)), it did not support growth even in animals given a daily supplement of 200 mg. per cent of choline chloride. The addition of oral doses of casein or of

dl-methionine brought about no improvement in the growth of the animals, nor did additional choline chloride or brewers' yeast. A liver extract fraction (Abbott's liver fraction "X") however, produced rapid and sustained growth when given as 4 per cent of the ration. Another liver extract low in choline (Wilson's liver fraction "L"), which is fairly potent in certain nutritional factors essential for the chick, supported growth when included at a 3 per cent level. Best growth was obtained in animals fed peanut flour in the place of peanut meal.

The development of the ration for the production of acute choline deficiency in weanling puppies permitted the investigators to estimate the choline requirement of the growing puppy and also to study some of the effects associated with choline deficiency. The choline requirement of the growing puppy on the ration used was judged to be not more than 100 mg. per 100 g. of ration or 50 mg. per kilogram of body weight per day.

Fatal deficiency of choline was produced in less than three weeks. This was characterized by severe fatty infiltration of the liver. The investigators found that 0.7 per cent dl-methionine or 0.1 per cent choline chloride supplemented to the ration seemed to render it adequate in all respects.

The changes found in the choline deficient animals were striking. The lipid content of the liver of these animals was extremely high (25.9 to 56.6 per cent on a dry weight basis) and was comparable to the fatty infiltration of the liver of the puppy with pantothenic acid deficiency (Schaefer, McKibbin, and Elvehjem, *J. Biol. Chem.* **143,** *321 (1942)*). In control animals receiving choline the range of lipid content was 7.4 to 20.5 per cent. In spite of the rise of total lipids, the cholesterol content of the liver was unaffected in the animals studied. The plasma phosphatase was increased several fold, and this seemed related to the degree of fatty infiltration of the liver.

The authors were able to demonstrate a marked impairment in the removal of bromsulfalein from the plasma of the dogs given no choline. The plasma from dogs receiving choline contained from 3 to 10 micrograms of bromsulfalein per milliliter of plasma after eight minutes whereas dogs receiving no choline had from 12 to 42 micrograms of the dye. The results of these tests, however, were not well correlated with the degree of fatty infiltration of the liver. In animals with livers containing large amounts of fat, significant increases in prothrombin time were found. No differences between deficient and control animals could be demonstrated by means of Gray's colloidal gold test.

Both cholesterol and cholesterol esters were reduced in the plasma of the animals deficient in choline. The total cholesterol and cholesterol esters of the plasma in the control animals ranged from 90 to 158 and from 57 to 95 mg. per cent, respectively. In the deficient animals the values were 40 to 83 and 9 to 58 mg. per cent respectively. A marked lowering in hemoglobin and volume of packed red cells was observed in 2 animals but the significance of this observation is difficult to determine since changes of corresponding degree were not noted in other animals.

Since creatine synthesis depends on the "methyl" pool, the blood creatinine and the muscle "total" creatine concentrations were determined but no changes were discovered. Total creatine excretion was not measured.

The changes which were noted in plasma phosphatase, cholesterol, and cholesterol ester values, as well as in bromsulfalein elimination and in prothrombin time are frequently seen in association with liver disease in man. McKibbin and associates assumed that the observed changes in these values did not reflect a primary biochemical defect of choline deficiency but represent a secondary effect incident to fatty infiltration of the liver. It would be of interest to know how the choline deficient pup responds to administration of choline as evidenced by changes in the various chemical measurements reported.

THE DESTRUCTION OF RIBOFLAVIN IN MILK BY LIGHT

Riboflavin in aqueous solution is rapidly destroyed by light, and as a result several investigators in recent years have studied the destruction of riboflavin in milk by light, in order to find whether significant losses may occur during the handling of milk by processors and distributors, or during cooking processes in the kitchen.

Williams and Cheldelin (*Science* **96,** *22 (1942)*) examined the question of loss of riboflavin during cooking. Their experiments were made in a well-lit room with an east window, during the afternoon when no direct sunlight entered the room. They exposed milk in glass tubes, maintained at a temperature of 100°C., at a distance of 5 feet from the window, and determined the riboflavin content of the milk before and after exposure. They found that the riboflavin was rapidly destroyed under these conditions; after five minutes the loss was 26 per cent; after fifteen minutes, 39 per cent; after thirty minutes, 48 per cent; and after forty-five minutes, 64 per cent. Heating the milk to 100°C. in the dark for forty-five minutes caused no significant loss of riboflavin.

Peterson, Haig, and Shaw (*J. Am. Chem. Soc.* **66,** *662 (1944)*) have turned attention to the fate of riboflavin in milk left standing in the bottle on the doorstep. They exposed milk in regular pint delivery bottles to late winter sunshine in North Carolina at a temperature of 15.5° to 22°C.; the bottles were allowed to stand on a porch in direct sunlight from midmorning to midafternoon. The riboflavin content of the milk specimens before and after exposure was measured. It was observed that the destruction in different bottles with different specimens of milk varied from 30 to 50 per cent of the original content.

Even larger losses were observed by Ziegler (*J. Am. Chem. Soc.* **66,** *1039 (1944)*) in a similar independent study conducted in Montreal. This investigator exposed milk in quart bottles to midmorning spring sunshine for one to two hours at temperatures from 16.7° to 20°C. In one hour the destruction in various bottles was from 26 to 36 per cent, and in two hours from 54 to 68 per cent. Thus serious loss of riboflavin occurs in bottled milk left outside the house and exposed to direct sunlight; and it is to be expected that considerable loss will occur in milk similarly exposed, not to direct sunlight, but to diffuse daylight. Such loss can be prevented by delivery of the bottle into a closed box, or by using bottles made of a suitable "non-actinic" glass.

The destruction of riboflavin during the processing of milk by the distributor has also been studied by Ziegler and Keevil (*J. Biol. Chem.* **155,** *605 (1944)*) who made measurements of the loss occurring during pasteurization and during irradiation with ultraviolet light for the enrichment of milk with vitamin D. During commercial pasteurization of milk it is heated in an exposed thin film, so that destruction might well occur. However, working under the actual lighting conditions of a dairy plant, Ziegler and Keevil found that the loss during pasteurization varied from 9 to 16 per cent in different batches. Milk samples taken before and after irradiation for vitamin D enrichment showed a loss of 5 to 8 per cent during this process. The effect of exposure to light during the bottling was also determined, and in this process there was a loss of from 3 to 5 per cent.

Reviewing these results, it appears that in general the destruction of riboflavin in milk by light is likely to be much more serious while it is in the bottle which has been delivered and left outside the house, than during processing or during cooking in the kitchen. As a practical measure to conserve the riboflavin of milk, apparently the most important factor is to see that milk bottles are not allowed to stand in the light outside the house after delivery.

NOTES

Vitamin K Action and Dicoumarin Antagonism

It is well known that several similar quinone-type compounds possess vitamin K activity, a fact which indicates that all of these may be converted to the same simpler active compound. Shemiakin and Schukina (*Nature* **154**, *513* (*1944*)) have reviewed their earlier work and present new evidence that the common active intermediate of the vitamin K quinones is phthalic acid.

The chemical structure peculiarities which give vitamin K compounds their antihemorrhagic activity appear to be the same which result in chemical instability leading to easy degradation to phthalic acid. This decomposition can occur *in vitro* by such mild treatment as warm water. Phthalic acid possesses some vitamin K activity, but its lower potency may be explained by a more rapid excretion. Vitamin K type quinones undergo a rapid change in the blood, although antihemorrhagic activity is evident only after a considerable lapse of time, presumably after phthalic acid formation. The more recent evidence in favor of the phthalic acid thesis consisted in the isolation of phthalic acid from the urine of man or the dog after administration of 2-methyl-1,4 napthoquinone (K_3). Vitamin K_3 itself was not found in the urine, while administered phthalic acid was excreted quantitatively and unchanged.

The authors also refer to the results of Link and co-workers (*Nutrition Reviews* **1**, *275* (*1943*)) who proposed that dicoumarin, a vitamin K antagonist, also owes its activity to degradation to a simpler compound, namely salicylic acid. These studies provide the simple attractive hypothesis that dicoumarin antagonism to vitamin K is due to an *in vivo* competition of two structurally similar molecules. Phthalic acid possesses two carboxyl groups on a benzene ring; salicylic acid one carboxyl and one hydroxyl group. The competition of structurally similar molecules acting either as substrates or coenzymes for enzyme systems is common knowledge among enzyme chemists and this principle has been further illustrated as a basis for production of vitamin deficiencies (*Nutrition Reviews* **2**, *59*, *101*, *320* (*1944*)).

Nutrition Problems in Venezuela

In Venezuela, as in many other parts of the world, attention is being given to the question of how the diet of the ordinary citizen can be improved. That the average Venezuelan diet is nutritionally suboptimal has been recognized for a long time, the three staples being black native beans, corn, and bananas. These three items are said to constitute the sole diet of many people for long periods of time, although fresh tropical fruits are consumed in large quantities in season. Meat, milk, and green vegetables are eaten rarely by the poorer people. Jaffé (*Revista de Sanidad y Asistencia Social* **8**, *1107* (*1943*)) has calculated the amounts of protein, fat, minerals, and vitamins in a typical mixture of the three foodstuffs sufficient to supply 3000 calories. Such a diet contained 140 g. of total protein, certainly a liberal amount, but the mixture nevertheless proved to be low in several of the essential amino acids. The most serious vitamin deficiencies appeared to be in vitamin A (1320 I.U.), vitamin C (24 mg.), and nicotinic acid (11.7 mg.). Calcium was suboptimal, especially for pregnancy and lactation. The question was raised whether there might not also be a deficiency in total calories in view of the large quantities of these foods that would have to be consumed to supply optimal amounts.

The specific nature of the deficiencies of the typical Venezuelan diet was demonstrated by feeding it to young weanling rats

(Jaffé, *Revista de Sanidad y Asistencia Social* **8,** *1117, 1129 (1943)*). Such rats grew much more poorly than those on a good stock diet, they failed to reproduce, and when the corn was white (as in many of the diets used by the people) symptoms of vitamin A deficiency appeared.

The addition to the basal diet either of casein or of a mixture of vitamins, produced a definite improvement in the growth rate, while dried milk produced a marked improvement. Guinea pigs fed the basal ration developed scurvy. Hemoglobin values, however, were normal in both species.

Attempts were next made to improve the diet in a practical way. The use of yellow corn instead of white corn, as would be expected, improved the growth rate and prevented the specific deficiency symptoms, although the addition of cod liver oil brought about further improvement, particularly when a better protein was supplied. The combination of yellow corn and soybeans, supplemented with minerals, produced good growth and reproduction that was considered satisfactory. Several varieties of soybeans were tested, and while they differed somewhat in the growth response elicited, they were all markedly superior to the common black bean. Accordingly the author recommends the more extensive cultivation and consumption of soybeans in place of black beans as a simple measure that would do much toward improving the nutrition of the general population. Several other types of food, particularly dairy products, would be most valuable additions to the dietary.

"Dietologia" and Alfalfa

A further example of the current worldwide interest in scientific nutrition is the appearance in Buenos Aires of a quarterly journal, "Dietologia," edited by Prof. Pedro Escudero of the Municipal Institute of Nutrition of that city. Each number of the journal contains a few reviews, many abstracts, and about a dozen original articles on each diverse subjects as gout, the chemical composition of dehydrated eggs, the vitamin content of foods, the effect of diet on intestinal organisms, and the nutritional adequacy of the meals served in boarding schools. Most of the articles compare favorably with those published in English. Each number also contains a biographical sketch of a contemporary scientist prominent in nutrition. Those described during the first year were Drs. Nelda Ross, J. deCastro, B. Houssay, and "Enrique" C. Sherman.

Two rather novel articles dealt with the possibility of using alfalfa in the human diet. The first (Escudero and Landabure, *Dietologia* **1,** *143 (1943)*) summarized the available information on the nutritive principles present in this plant, which can be cultivated for at least six months in most parts of Argentina. It is pointed out that in terms of protective factors this food costs less than any other that is available. The second paper (Escudero and Falabella, *Ibid.* **1,** *148 (1943)*) deals with specific suggestions as to how 50 g. of alfalfa can be incorporated into the daily diet. It is recommended that only the upper parts of young plants that have not yet reached the flowering stage be used. Older plants contain more indigestible material. Nine recipes are included for such items as alfalfa salad (washed, separated, and served with oil, salt, and a dash of lemon or vinegar), alfalfa tortillas, cream of alfalfa soup, alfalfa croquettes, and alfalfa soufflé. The authors do not state whether they themselves make use of these recipes, and in any event the response of the Argentine population will be awaited with interest.

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